IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
Wolfgang M. Franz et al.
Serial No. 09/068,751
filed: November 14, 1996
for: Gene Therapeutic Nucleic Acid Working Model and Its Production and Use for Treating Heart Disease

DECLARATION UNDER RULE 132

I, the undersigned, Wolfgang M. Franz, a citizen of Germany, do hereby depose and declare:

I have graduated in medicine and I was awarded the degree of PhD from the Technical University of Munich, Germany, in 1986.

My major research interest is directed to gene therapy of cardiovascular diseases.

I am co-inventor of the subject matter of U.S. Patent Application No. 09/068,751.

I have carefully read the Office Action dated December 20, 2001 and the specification of the present patent application.

With regard to the Examiner's contention that the specification does not enable any person skilled in the art to make and / or use the invention commensurate in scope with the present claims I would like to provide the following response:

The present application is, to my opinion, the first document disclosing the successful cardiac specific, in particular ventricle specific, gene transfer in neonatal or adult animals by making us of viral vector systems.

In my opinion, a skilled person would have been enabled by the detailed disclosure of the specification to perform the invention, in particular the tissue specific gene transfer in adult or neonatal animals.

The experiments disclosed in the specification illustrate operability of a **functional** regulatory nucleic acid sequence as obtained from the **rat** mlc-2 gene under conditions of somatic gene transfer (see in particular Examples 8 to 11 of the specification).

Meanwhile we were able to locate the corresponding regulatory sequence of the **human** mlc-2 gene. The obtained nucleotide sequence is attached as

ANNEX I

We have analysed the sequence for potential regulatory elements and could locate sequence motifs **almost identical** to those disclosed in the present specification and considered as vital for the intended purpose. These elements are, in 5'-3' direction, the following: CSS-like sequence, MLE1-box, HF-1a-, HF1b-, HF-2-, HF-3- and E-box. The same sequence motifs have been identified in the specification to play a critical or at least favourable role in the regulation of cardiac specific gene expression.

Said high degree of similarity observed between rat and human regulatory elements is illustrated by the partial sequence alignment attached as

ANNEX II

The rat sequence motifs are shown below the corresponding human sequence motifs. Identical nucleotide residues are identified by a vertical line between both sequences.

On the basis of said additional xperimental evidence, I am convinced that a skilled person will not have to practice "trial and error" experimentation in order to provide

viral constructs other than those exemplified in the specification which can be successfully used for cardiac specific, in particular ventricle specific, gene transfer.

I would also expect that a similar favourable cardiac tissue specificity is also observed when the same regulatory elements are inserted in different viral vectors, because cardiac tissue specificity, which, in my opinion is the key for successful and valuable somatic gene transfer to the heart muscle, is mainly influenced by the cardiac tissue specificity of the promoter sequence operatively linked to the gene to be expressed with cardiac specificity.

In this respect, I would like to point to a comparative experiment already disclosed in the present specification. Example 11 describes the differences in cardiac tissue specificity and activity of gene expression observed on the one hand for a recombinant virus vector construct of the present invention, i.e. **Ad-mlcLuc** , and, on the other hand, for a virus construct carrying, **in the same virus, a different muscle derived promoter**, i.e. alpha-mhc. Said construct is designated **Ad-mhcLuc**. As illustrated by Figures 8A and 8B merely Ad-mlcLuc, i.e. the viral construct of the present invention, provides for a strong and cardiac specific gene expression while Ad-mhcLuc also directs non-specific gene expression. Significant levels of non-specific gene expression were observed for Ad-mhcLuc in kidney, spleen, liver, diaphragm, lung and intercostal muscle. Moreover, the construct of the invention was three to four times more active in the heart than Ad-mhcLuc. Said experimental evidence supports my point of view that a skilled reader of the present specification will in fact be able to practice, without the need of "trial and error" experimentation, the invention within the scope of the claimed recombinant vectors, carrying a regulatory mlc-2 gene fragment which must be functional, i.e. directs cardiac specific gene expression.

The undersigned declares further that all statements made herein on his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the lik so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and

that such wilful false statements may jeopardise the validity of this application or any patent issuing thereon.

Munich, 6-17-2002

(Wolfgang M. Franz)